Asian J. Exp. Sci., Vol. 18, No. 1&2, 2004, 37-46

Effect of Fluoride Contaminated Drinking Water in Albino Rats *Rattus norvegicus*

J.D Sharma, M.K Sharma and P. Agrawal
Reproductive Physiology and Environment Toxicology Laboratory,
Department of Zoology,
University of Rajasthan,
Jaipur- 302004, India

Abstract : Healthy, adult albino rats were treated with fluoride water (1.5, 3, 4.5, 6 ppm) for 60 days. The data reveal that reduction in weight of kidney was observed after the ingestion 3 and 6 ppm of fluoride water. The haematological parameters found to be altered with higher dose of fluoride water leading to anaemic condition. The serum protein, cholesterol and phospholipids were reduced in all the groups studied. However no change was observed in enzyme activity of serum alkaline phosphatase. The tissue biochemistry (glycogen, cholesterol, ascorbic acid) of liver, heart, kidney and adrenal diminished following fluoride water treatment. The data suggests that excess fluoride water exposure to rats caused reduction in weight of kidney, altered serum and tissue biochemistry in turn causing toxic effects on liver, heart, kidney and adrenal.

Introduction

Water pollution has become world wide phenomenon. The under ground water is polluted by many hazardous pollutants like coloured dyes, nitrates, heavy metals, pesticides and fluoride. Fluoride is one of the major concern among these pollutants. The problem due to high concentration of fluoride in ground water has now become one of the most important health related geo-environmental issues in India.

Fluoride is an essential ion for human being from health point of view. According to World Health Organization (1984), permissible limit of fluoride in drinking water is 1.5 ppm. All the districts of Rajasthan State are affected with higher concentration of fluoride in drinking water which leads to various health problem *viz.* stiffening of body joints, deformation of bones and teeth mottling called fluorosis. (Mathur, 1983) analyzed the drinking water fluoride contents in major districts of Rajasthan and noticed that the fluoride level exceeds to 1.5 ppm and goes to maximum upto 10 ppm. People residing in areas having high fluoride content manifest dental and skeletal fluorosis over a period of time (Susheela *et al*., 1984). Even the ingestion of soil with high fluoride concentration may cause chronic

fluorosis in grazing animals (Loganathan *et al*., 2001). The fluoride toxicity in heart muscle of rabbits have been reported (Shashi and Thapar, 2001), yet a paucity of literature on fluoride toxicity on soft tissues in animals prevails. Therefore, the present investigation has been undertaken to focus on the effect of fluoride contaminated drinking water in rats.

Materials and methods

The fluoride contaminated drinking water samples were obtained from villages of Sanganer Tehsil and analyzed for fluoride content using standard technique from Malviya National Institute of Technology. The above samples of different fluoride concentrations (1.5, 3, 4.5 and 6 ppm) were used for experiments.

Healthy, adult albino rats *Rattus norvegicus* weighing between 200 to 250 gm were used for experiments. They were housed in an air-cooled animal house at a temperature of $26^0 \pm 2^0$C and exposed to 14h of daylight. The animals were divided into five different groups containing six rats in each group. Group I- control received only tap water. Group II, III, IV and V were treated with fluoride water 1.5 ppm, 3 ppm, 4.5 ppm and 6 ppm, respectively for 60 days. The animals were maintained on standard diet (Hindusthan lever ltd.,) and water was given *ad libitum*. The animals were sacrificed by cervical dislocation, and blood was extracted through cardiac puncture. The liver, kidney, heart and adrenal gland were carefully dissected out, blotted free of blood, weighed and used for various parameters using standard techniques. Total erythrocytes and total leucocytes count (Wintrobe, 1930), percent haemoglobin (Crossby *et al*., 1954) and haematocrit (Strumia *et al.*, 1954) values were studied in blood. The total protein (Lowry *et al*., 1951), cholesterol (Zlatkins *et al.,* 1953), phospholipid (Zilversmit *et al.*, 1950) and alkaline phosphatase (Fiske and Subbarow, 1965) were estimated in serum. The estimation of cholesterol (Oser, 1965), glycogen (Montgomery, 1957) and ascorbic acid (Roe & Kuther, 1943) were done in liver, heart and adrenal. A minimum of six replicates were done for each tissue and treatment. The results were statistically analyzed using Student's 't' test.

Result

The data revealed that fluoride treatment to rats did not bring any significant change in the body weight, but the vital organ weights were declined in all the group studied. The significant reduction was observed in kidney with 3 ppm and 6 ppm treatment for 60 days. Almost significant reduction was noted in weight of heart following fluoride water 3 and 6 ppm treatment as compared to control value (Table-I).

Fluoride water treatment to rats resulted in significant ($p<0.001$) reduction of total erythrocyte, haemoglobin percentage and haematocrit value in all the groups studied. However there was significant ($p<0.001$) increased in total leucocyte count was noted with fluoride water exposure in group III, IV and V (Table-II).

The protein, cholesterol and phospholipid content of serum diminished significantly ($p<0.001$) following fluoride water treatment in G-II to V except the phospholipid content of G-II wherein non significant change was noted as compared to control value. However no significant change in enzyme activity of alkaline phosphatase was observed throughout the experimentation (Table-III).

The glycogen concentration of liver declined significantly ($p<0.05$) with higher dose of fluoride content in water as compared to control value, but there was non-significant change was observed in heart in all the groups studied. The decline in cholesterol content was observed in heart muscle in G- II to V, liver in G- III to V and adrenal showed non significant change with all dose level of fluoride water exposure to rats as compared to control group. However ascorbic acid mobilization was observed in adrenal following fluoride water (1.5, 3, 4.5 and 6 ppm) treatment to rats leading to significant reduction ($p<0.001$) as compared to control group (Table-IV).

Discussion

In the present study the weight of kidney, heart and adrenal declined which may be due to a direct effect of fluoride water on vital organs. The ingestion of a high fluoride content results in a decrease in food intake and reduction in growth rate of the rodents in mammals (Simon and Suttie 1968; Weber and Reid 1969).

In the present investigation lower haemoglobin concentration and RBC count observed in rat which is in conformity with the Kahl *et al*., (1973) and Pillai and Mane, (1985). A decline in RBC, haemoglobin and haematocrit values in 20 ppm fluoride treated rats have been reported (Banu Priya *et al*., 1997). As well as increased WBC count in rats is in concord with these finding. Fluoride depletes the energy reserves and the ability of WBC to properly destroy foreign agents by the process of phagocytosis in turn reduced immunity leading to general weakness in animals. (Gabler and Leong, 1979; Gabler *et al*., 1985 and Kozlyuk *et al*., 1987). Serum protein, cholesterol and phospholipid concentration decrease following fluoride treatment to rats.

The altered tissue biochemistry of liver, heart and adrenal may be due to toxic effects of fluoride on vital organs because ingestion of fluoride causes decrease in the ionized calcium (Teotia and Teotia, 1972; Gupta, 1999; Srivastava *et al.,* 1989). This hypocalcemia lead to change in internal milieu of the body to maintain the calcium level and lead to secondary hyperparathyroidism. It was well known that ionic calcium was one of the important ions for the initiation and maintenance of the activity of the vital organs and musculo-skeletal system. Lowering of the ionized calcium was one of the important stimulus for the release of PTH (Schwartz *et al*., 1998). Increased parathyroid hormone causes increased activity of osteoclasts in bone by activating membrane bound 3’5' cyclic AMP (Tortora *et al*., 1990). Therefore, the present finding suggests that excess fluoride ingestion to rats caused adverse effects on soft tissues, hampering normal physiology of liver, heart, kidney and adrenal.

Acknowledgement

Authors are thankful to SAP and HOD Dept. of Zoology, University Rajasthan, Jaipur for providing necessary facilities.

TABLE I : Body (g) and Organ weights (mg/100 g body wt.) of control and fluoride contaminated drinking water treated rats for 60 days

Group	Treatment		Body Weight (g)		Vital Organ (mg)			
			Initial	Final	Kidney	Liver	Heart	Adrenal Gland
I	Control		231.20 ±12.40	297.00 ±60.00	749.35 ±13.15	3141.35 ±138.75	367.02 ±36.65	23.02 ±1.9
II	Fluoride Contaminated drinking water for 60 days	1.5 ppm F	262.00 ±15.00	287.00 ±5.0	726.41 ±19.64	3274.22 ±61.11	377.49 ±15.27	22.22 ±0.78
III		3.0 ppm F	270.53 ±2.40	313.00 ±5.0	662.41[a] ±18.48	3132.18 ±55.40	273.49[c] ±18.99	21.29 ±1.49
IV		4.5 ppm F	265.33 ±4.40	285.66 ±6.70	742.06 ±36.69	3247.14 ±220.84	340.11 ±20.41	20.17 ±1.62
V		6.0 ppm F	262.00 ±7.50	311.5 ±10.00	684.18[b] ±6.03	3150.50 ±64.42	302.03 ±2.03	19.47 ±0.68

Values are mean ± SE

c = $p \leq 0.05$ almost significant b = $p \leq 0.01$ significant a = $p \leq 0.001$ Highly significant

TABLE II : R.B.C., W.B.C., Haemoglobin and Haematocrit values of control and fluoride contaminated drinking water treated rats for 60 days

Group	Treatment		Blood			
			R.B.C. (million/mm^3)	W.B.C. (per mm^3)	Haemoglobin (gm%)	Haematocrit (%)
I	Control		5.50 ±0.37	8502.50 ±183.01	13.90 ±1.05	46.33 ±0.79
II	Fluoride Contaminated drinking water for 60 days	1.5 ppm F	3.42^a ±0.03	8713.13 ±28.36	13.26 ±0.10	42.73 ±0.31
III		3.0 ppm F	2.99^a ±0.01	8924.93 ±26.71	10.29^b ±0.08	38.11^a ±0.07
IV		4.5 ppm F	2.97^a ±0.02	9051.75^b ±65.39	9.76^a ±0.08	33.45^a ±1.27
V		6.0 ppm F	2.06^a ±0.04	10646.02^a ±156.34	9.12^a ±0.04	24.59^a ±0.62

Values are mean ± SE

c = $p \leq 0.05$ almost significant b = $p \leq 0.01$ significant a = $p \leq 0.001$ Highly significant

TABLE III : Total Protein, Cholesterol, Phospholipid and Alkaline Phosphatase in serum of control and fluoride contaminated drinking water treated rats for 60 days

Group	Treatment		Total Protein (mg/dl)	Cholesterol (mg/dl)	Phospholipid (mg/dl)	Alkaline Phosphatase (µg ip/hr.)
I	Control		16878.64 ±320.56	109.40 ±0.90	154.60 ±3.61	3.16 ±0.03
II	Fluoride Contaminated drinking water for 60 days	1.5 ppm F	14755.92[a] ±72.57	87.86[a] ±0.26	150.30 ±2.18	3.14 ±0.01
III		3.0 ppm F	14058.94[a] ±29.03	87.050[a] ±1.38	103.12[a] ±7.47	3.15 ±0.02
IV		4.5 ppm F	13418.61[a] ±196.15	67.89[a] ±4.68	95.02[a] ±1.50	3.14 ±0.02
V		6.0 ppm F	13020.49[a] ±27.14	54.42[a] ±0.97	79.31[a] ±0.36	3.15 ±0.02

Values are mean ± SE
c = $p \leq 0.05$ almost significant

TABLE IV : Glycogen, Cholesterol and Ascorbic acid in tissues of control and fluoride contaminated drinking water treated rats for 60 days

Group	Treatment		Glycogen (mg/gm)		Cholesterol (mg/gm)			Ascorbic Acid (mg/gm)
			Liver	Heart	Liver	Heart	Adrenal Gland	Adrenal Gland
I	Control		7.54 ±0.68	2.85 ±0.05	9.48 ±0.48	6.05 ±0.15	25.45 ±1.35	3.45 ±0.11
II	Fluoride Contaminated drinking water for 60 days	1.5 ppm F	7.41 ±0.04	2.69 ±0.02	9.47 ±0.01	4.38[a] ±0.07	23.40 ±0.02	2.77[a] ±0.01
III		3.0 ppm F	6.27[c] ±0.08	2.46 ±0.02	9.22 ±0.01	4.82[a] ±0.01	23.39 ±0.03	2.59[a] ±0.01
IV		4.5 ppm F	5.97[c] ±0.01	2.27 ±0.03	8.32[c] ±0.21	3.07[a] ±0.02	23.47 ±0.01	2.55[a] ±0.02
V		6.0 ppm F	5.85[c] ±0.02	2.22 ±0.02	7.85[c] ±0.04	2.71[a] ±0.02	23.21 ±0.01	2.50[a] ±0.02

Values are mean ± SE

c = $p \leq 0.05$ almost significant a = $p \leq 0.001$ Highly significant

Reference

Banu Priya C.A.Y., Anitha K., Muralimahon E., Pillai K.S. and Murhty P.B. (1997) : Toxicity of fluoride to diabetic rats. *Fluoride* **30 (1),** pp 51-58.

Crossby W. H., Munn J. I. and Funth F.W. (1954) : Standardizing. A Method for clinical haemoglobin Met. Ery. U.S. Armed Force Med. J. S.: 695-703.

Fiske C.M. and Subbarow Y. (1965) : Method given by Hawk, P.G, Oser, B.L. and Summerson, W.H.(1965). Practical Physiology Chemistry, (4th Ed.), Mc Grew Hill Book Co., New York.

Gabler W.L. (1985) : Effect of fluoride on the kinetics of superoxide generation by fluoride. *Journal of dental Research*, **64,** 281-283.

Gabler W.L. and Leong P.A. (1979) : Fluoride inhibition of poly morphonum clear leukocyte. *Journal of dental Research*, **48(9),** 1933-1939.

Gupta S.K. (1999) : "Environmental Health Perspective of Fluorosis in Children" (Ph. D Thesis), Jaipur, Rajasthan: University of Rajasthan, Jaipur.

Kahl S., Wojcik Ewy Z. (1973) : Effect of fluoride on some haematological indices and iron-59 distribution in the blood and iron storing tissues of rats. Bulletin of Academy of Poland Science Series in Biology **21,** 389-393.

Kozlyuk A.S. (1987) : Immune status of children in chemically contaminated Environments. Dravookhra nenic issue **3,** 6-9.

Loganathan P., Hedly M.J., Wallace G.C. and Roberts A.H. (2001) : Fluoride accumulation in pasture forages and soils following long term application of phosphorous fertilizers. *Eviron. Pollut.*, **115(2),** 275-282.

Lowry O.H., Rosenbrough M. J., Farr A.L. and Ranall R.J. (1951) : Protein measurement with the folin phenol reagent. *J. Bio. Chem*., **193,** 265-275.

Mathur S.C. (1983) : Endemic fluorosis in Rajasthan India Association of preventive and social Medicine, Raj. 3rd conference 21-22 Oct. 1983. Sardar Patel Medical Collage. Bikaner.

Montgomery R. (1957) : Determination of glycogen. *Arch. Biochem. Biophys*. **67,** 378-389.

Oser B. L. (1965) : In Hawk's Physiological Chemistry. (14th ed.) McGraw Hill, New York, 246-249.

Pillai K.S. and Mane U.H. (1985) : Effect of airbome fluoride on some haematological parameters of chik. Bulletin of environment contamination and Toxicology. **33,** 510-516.

Roe J.H. and Kuether C.A. (1973) : The determination of ascorbic acid in whole blood and urine through the 2-4-di-nitro-phenyl hydrazine derivative of dihydro ascorbic acid. *J. Biol. Chem*., **147,** 399-407.

Schwartz P., Madsen J.C., Razsmussen A.Q., Transbd I.B. and Brown E.M. (1998) : Evidence for a role of intracellular stored parathyroid hormonic in producing hysteresis of PTH. Calcium relationship in normal humans. *Clinical Endocrinology*. **48,** 725-732.

Shashi A. and Thapar S.P. (2001) : Histopathology of mercurial damage in experimental fluorosis in rabbits. *Fluoride* **34(1),** 43-50.

Simon G. and Suttie J.W. (1968) : Effect of dietary fluoride on food intake and plasma fluoride concentration in the rat. *J. Nutrition* **96,** 152-154.

Srivastava R.N.X, Gill D.S., Moudgil A., Menon R.K., Thomas M. and Dandona P. (1989) : Normal ionized calcium, Parathyroid hypersecretion, and elevated Osteocalcin in a family with Fluorosis.Metabolism, **38(2),** 120-124.

Strumia M.M., Sample A.B. and Hart E.D. (1954) : An improved micro haematocrit method. Am. *J. Clin. Pathol*. **24,** 1016-1024.

Susheela A.K., Jha Mohan, Koacher, Jasbeer and Jain S.K. (1984) : Fluoride and calcified Tissues.In: Fluorosis Research Strategies. African Medical and Research Foundation Narrobi, Kenya.

Teotia S.P.S. and Teotia M. (1972) : Hyper activity of the parathyroid glands in endemic osteofluorosis, *Fluoride*, **5,** 115-117.

Tortora J.G. and Anagnostakas N.P. (1990) : Skeletal tissue: Homeostasis of remodeling, In: Principles of Anatomy and Physiology. Harper & Row Publishers, New York , pp150-151.

Weber C.W. and Reid B.L. (1969) : Fluoride Toxicity in the mouse. *J. Nutrition*, **97,** 90-96.

WHO World Health Organization, 1984 : Environmental Guidelines by WHO for drinking water quality, 1984: vol. 1-3, Geneva.

Wintrobe M.M. (1930) : A simple and accurate haematocrit. *J. Lab. Clin. Med*. **15,** 287-289.

Zilversmit D.B., Davis A.K. and Melpmphils B.S. (1950) : Micro determination of plasma phospholipid by dichloraceticacid precipitaion. *J. Lab. Clin. Chemist*. **35,** 155-160.

Zlatkis A., Zak B. and Boyle A. J. (1953) : A new method for direct determination of serum cholesterol. *J. Lab. Clin. Med*. **41,** 486-92.